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Possible therapeutic effects of boron citrate and oleoylethanolamide supplementation in patients with COVID-19: A pilot randomized, double-blind, clinical trial

Neda Akbari [a], Alireza Ostadrahimi [b], Helda Tutunchi [a], Samira Pourmoradian [b], Nazila Farrin [b], Farzad najafipour [a], Hamid Soleimanzadeh [c], Behnam Kafil [d], Majid Mobasseri [a,*]

[a] Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Nutrition Research Center, Department of Clinical Nutrition, School of Nutrition & Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Department of Applied Chemistry, Faculty of Chemistry, University of Tabriz, Iran
[d] Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Background: The present study aimed to assess the therapeutic effects of boron citrate and oleoylethanolamide supplementation in patients with COVID-19.
Methods: Forty adult patients with a diagnosis of COVID-19 were recruited in the present study. Patients were randomized in a 1:1:1:1 allocation ratio to 1of 4 treatment groups: (A) 5 mg of boron citrate twice a day, (B) 200 mg of oleoylethanolamide twice a day, (C) both therapies, or (D) routine treatments without any study medications. At pre-and post-intervention phase, some clinical and biochemical parameters were assessed.
Results: Supplementation with boron citrate alone or in combination with oleoylethanolamide significantly improved O2 saturation and respiratory rate ($p < 0.01$). At the end of the study, significant increases in white blood cell and lymphocyte count were observed in the boron citrate and combined groups ($p < 0.001$). Boron citrate supplementation led to a significant decrease in serum lactate dehydrogenase ($p = 0.026$) and erythrocyte sedimentation rate ($p = 0.014$), compared with other groups. Furthermore, boron citrate in combination with oleoylethanolamide resulted in a significant reduction in the high-sensitivity C-reactive protein and interleukin-1β concentrations ($p = 0.031$ and $p = 0.027$, respectively). No significant differences were found among four groups post-intervention, in terms of hemoglobin concentrations, platelet count, and serum interleukin-6 levels. At the end of the study, common symptoms of COVID-19 including cough, fatigue, shortness of breath, and myalgia significantly improved in the supplemented groups, compared to the placebo ($p < 0.05$).
Conclusion: Supplementation with boron citrate alone or in combination with oleoylethanolamide could improve some clinical and biochemical parameters in COVID-19 patients.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late December 2019 and has caused a pandemic of acute respiratory disease, named 'coronavirus disease 2019′ (COVID-19), which threatens human health [1]. This infection usually leads to a defined pattern of metabolic and clinical changes in patients [2]. Most common symptoms of COVID-19 infected patients are fever, cough, breathing problems, sore throat, unexplained loss of taste or smell, myalgia, palpitations, headache, and finally severe respiratory syndrome [3]. The pandemic of COVID-19 presents an unprecedented challenge to identify effective medication therapy strategies for prevention and treatment [4]. Most of the treatment options available for COVID-19 are based on previous experiences in treating severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) coronaviruses [5]. In addition, new evidence indicates that therapeutic agents with antiviral properties as well as those with anti-inflammatory and immunomodulatory activities may be effective in managing COVID-19 infection [6,7].

Boron, an intriguing element in the periodic table, is an important

* Corresponding author.
E-mail address: mobasserimajid@yahoo.com (M. Mobasseri).

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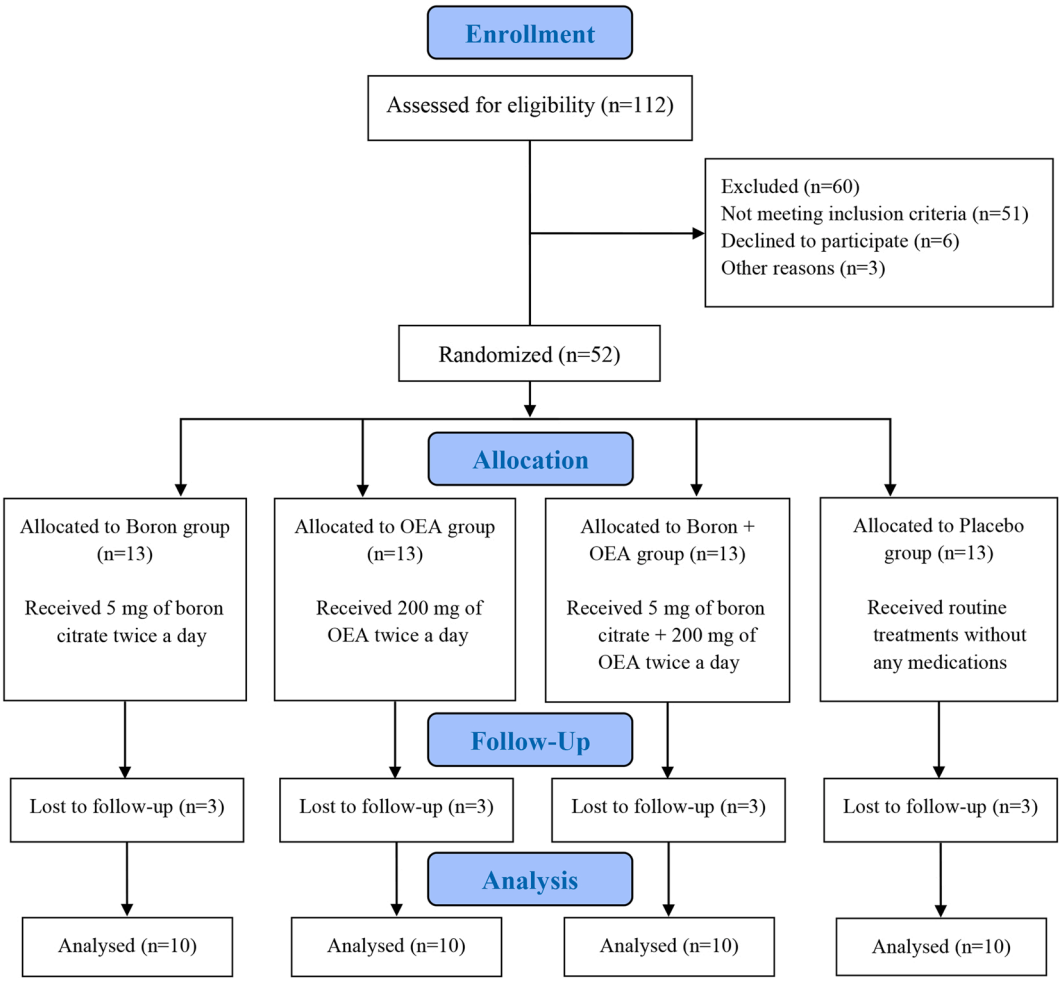

Fig. 1. Study flow diagram. OEA, Oleoylethanolamide.

trace element that plays a major role in biological functions [8,9]. Boron is involved in hormone and mineral metabolism and has also been reported to modulate inflammatory responses in animals and humans. Moreover,boron shows antioxidant activity by inhibiting production of reactive oxygen species (ROS) [10,11]. Boron exists in the body mainly in the form of boric acid (BA), a serine protease inhibitor. BA is one of the most important boron compounds used in agriculture, medicine, and pharmacology. Furthermore, BA is used in the preparation of many boron compounds including boron nitrite, boron phosphate, and organic boron chemicals, such as boron citrate and borax. Compared with inorganic acids, organic acids exert several benefits, for instance, they can perform the reaction under weak acidic conditions, and also the production cost of them is low [12,13].

Boron-containing compounds have recently gained growing interest as potential novel therapeutic agents due to a wide range of biological activities, including antibacterial, antifungal, antiviral, and anti-inflammatory activities [14–18]. The effect of BA on the differentiation of lymphocyte clusters in mice and rats has been reported [19,20]. The oral administration of borax exhibited a significant increase in T and B-cell populations as well as an increase in CD4 and CD19 cells in mice [19]. Previous work conducted by Jin et al. [20] in rats indicated that administration of 20 and 40 mg/L of boron let to an increase in the level of serum immunoglobulin G (IgG), the number of leukocytes, erythrocytes, lymphocytes, and monocytes, and splenic CD3 + T cells, and also improved non-specific and specific immune responses. The anti-inflammatory activity of certain natural and synthetic boron-containing compounds has also been reported in infectious disease [21].

Emerging evidence indicates that boron-based drugs can also be considered as viral protease inhibitors. These compounds showed potent enzyme inhibitory activity [22,23]. The administration of these compounds on humans with virus infections has led to attractive results such as inhibition of severe fever with thrombocytopenia syndrome virus or prevention of influenza virus growth [24]. For example, bortezomib, the boron-based drug, is the clinically approved proteasome inhibitor with demonstrated antiviral activity. This drug could significantly inhibit growth of the influenza virus in Madin-Darby canine kidney and human A549 lung cells [25]. Boromycin, the first natural compound containing boron, is reported to inhibit the replication of the clinically isolated human immunodeficiency virus type 1 (HIV-1) strain as well as the cultured strain in in vitro [26]. It is also found that boron can enhance the antiviral activity of the curcumin against SARS-CoV-2 [27]. In general, it has been suggested that boron compounds may be effective against SARS-CoV-2 due to their antiviral properties [28].

In recent years, peroxisome proliferator-activated receptor-alpha (PPAR-α), a ligand-activated transcription factor, has emerged as a useful drug target for the regulation of pathophysiological functions, including inflammation and oxidative stress [29]. PPAR-α has been shown to negatively regulate pro-inflammatory and acute phase response signaling pathways [30,31]. The bioactive lipid oleoylethanolamide (OEA), is an endogenous high-affinity agonist of PPAR-α. Previous studies have demonstrated that OEA is a potent anti-inflammatory and antioxidant agent [32,33]. The positive correlation between the cytokine storm and the severity of COVID-19 is reported [34]. OEA has been shown to bind to PPAR-α, leading to a cascade of events that can ultimately suppress inflammatory responses

Table 1
General Characteristics of the Study patients.

	Boron (n = 10)	OEA (n = 10)	Boron + OEA	Placebo (n = 10)	P value
Age (y)	44.10 (6.67)	38.90 (7.82)	43.80 (13.82)	44.40 (7.93)	0.525[a]
Male, n (%)	4 (40)	2 (20)	5 (50)	4 (40)	0.567[b]
Severity of disease at baseline, n (%)					
Mild	6 (60)	7 (70)	5 (50)	6 (60)	0.841[b]
Moderate	4 (40)	3(30)	5 (50)	4 (40)	
Common COVID-19 symptoms at baseline, n (%)					
Fever	7 (70)	9 (90)	7 (70)	8 (80)	0.665[b]
Cough	9 (90)	9 (90)	9 (90)	9 (90)	1.000[b]
Fatigue	9 (90)	10 (100)	8 (80)	9 (90)	0.528[b]
Shortness of breath	8 (80)	9 (90)	8 (80)	8 (80)	0.915[b]
Sore throat	3 (30)	3 (30)	2 (20)	4 (40)	0.813[b]
Myalgia	8 (80)	6 (60)	8 (80)	9 (90)	0.590
BMI (kg/m^2)					
Before	24.47 (3.71)	23.83 (3.70)	24.63 (3.95)	25.22 (2.61)	0.853[a]
After	24.02 (3.55)	23.29 (3.30)	24.02 (3.23)	25.36 (2.46)	0.073[d]
MD (95% CI)	0.45 (−0.35, 1.25)	0.54 (−0.21, 1.29)	0.61 (0.03, 1.18)	-0.14 (−0.55, 0.27)	
P value[c]	0.237	0.139	**0.041**	0.467	

BMI, body mass indez; MD, mean difference; OEA, Oleoylethanolamide.
Numerical data are presented as Mean (SD); categorical variables are presented as number (%).
[a] One-way analysis of variance (ANOVA).
[b] Chi-square ($\chi 2$) test.
[c] Paired-samples *t*-test.
[d] ANCOVA adjusted for age, sex, and baseline values.

[35].

We hypothesized that boron citrate and OEA supplementation may be effective in COVID-19 patients due to their anti-inflammatory, antioxidant, and immunomodulatory activities. The present clinical trial aimed to examine possible therapeutic effects of boron citrate and OEA supplementation in patients with COVID-19.

2. Methods

2.1. Study design

This single-center, four-armed parallel-group, randomized double-blind clinical trial was performed at outpatient clinic of Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran. Forty adult patients with a diagnosis of COVID-19 confirmed by polymerase chain reaction (PCR) assay or chest CT scan were recruited in the present study, conducted between May to Jun, 2020.

Patients (or their legally authorized representative) who were willing and able to provide written informed consent prior to study were included. The following were the exclusion criteria: severe COVID-19 pneumonia, advanced chronic kidney disease, severe liver disease, human immunodeficiency virus (HIV) infection or other immunodeficiencies, cancer, ischemic myocardial disease, thromboembolic disease, pregnancy or breastfeeding, receipt of any experimental treatment for COVID-19 within the 30 days prior to molecular screening, and regular consumption of any type of supplement.

Ethical approval for the present study was obtained from the Local Research Ethics Committee (IR.TBZMED.REC.1399.099). The present trial was registered at clinicaltrials.gov as IRCT20090609002017N35 and conducted in accordance with the Helsinki Declaration.

3. Randomization and intervention

After obtaining the informed consent and expressing the objectives of the research, eligible patients (n = 40) were divided into four equal groups, using the Random Allocation Software (RAS). The block randomization was conducted by an assistant and the intervention allocation was blinded for both the investigators and participants. Patients were randomized in a 1:1:1:1 allocation ratio to 1 of 4 treatment groups with a treatment duration of two weeks after a positive diagnosis. The 4 treatment groups were as follows: (A) 5 mg of boron citrate twice a day, (B) 200 mg of OEA twice a day, (C) both therapies, or (D) routine treatments including the use of antiviral and antibiotic drugs, providing nutritional support, without any study medications. The person completely unrelated to the study was asked to assign a three-digit code to each of the capsules. All capsules were identical in shape, size, and color. They were synthesized at the Nutrition Research Center of Tabriz University of Medical Sciences, Iran.

4. Assessments

All assessments were performed at baseline and after 14 days of the intervention. Data on age, weight, height, and medications were collected using medical records. The common symptoms of COVID-19 including respiratory rates (RR) as well as dry cough, fever, fatigue, sore throat, and myalgia of the patients were recorded by a physician and O2 saturation (O2sat) was measured using a pulse oximeter.

Blood samples (5 ml) were collected following a 12-h overnight fast and centrifuge at 3000 g for 5 min to extract serum samples. Complete blood count (CBC), the erythrocyte sedimentation rate (ESR), and serum lactate dehydrogenase (LDH) were measured using an automated hematology analyzer, the Westergren and enzymatic methods, respectively. Serum levels of inflammatory markers including high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)−1β and IL-6 were assessed using enzyme-linked immunosorbent assay (ELISA) kits (crystal day, Shanghai, China) according to the manufacturer's instructions.

5. Statistical analysis

All statistical analyses were conducted using SPSS statistical software (SPSS Inc., Chicago, IL, USA, version 23). The Kolmogorov-Smirnov test was used to assess the normal distribution of variables. Data were presented as mean ± standard deviation (± SD) and median (25th–75th percentile) for normally and non-normally distributed quantitative variables, respectively, with frequency (percentage) for qualitative data. For comparison of quantitative variables across four arms of the study, one-way analysis of variance (ANOVA) or non-parametric Kruskal–Wallis test were applied. We used Chi-square ($\chi 2$) test to compare categorical variables across four arms of the study. Tukey's post hoc was done to analyze the multiple comparisons. Comparison of the 4 groups at the end of the study was completed by analysis of covariance (ANCOVA) followed by Sidak's test after adjusting for confounding variables (baseline value of each item and gender). To compare baseline with final results within each group, paired samples *t*-test and non-parametric Wilcoxon signed-rank test were applied. P values less than 0.05 were considered statistically significant.

6. Results

Out of the 52 patients who enrolled in the present study, 40 completed the trial while 3 patients in each arm discontinued the study. Fig. 1 presents the study flowchart. No adverse effects or symptoms following supplementations were reported by the patients. At baseline, there were no statistically significant differences among four groups in

Table 2
Comparison of clinical and biochemical parameters among patients throughout the study.

	Boron (n = 10)	OEA (n = 10)	Boron + OEA	Placebo (n = 10)	P value
O2 saturation (%)					
Before	87.20 (2.89)	87.50 (3.83)	88.00 (3.59)	86.60 (3.16)	0.827[a]
After	92.00 (1.56) *	90.90 (2.99)	92.10 (2.72)[#]	89.20 (2.14) *,[#]	**< 0.001**[b]
MD (95% CI)	-4.80 (−6.09, −3.50)	-3.40 (−4.42, −2.37)	-4.10 (−5.33, −2.86)	-2.60 (−3.62, −1.57)	
P value[c]	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	
Respiratory rate (breaths/min)					
Before	22.50 (2.01)	22.00 (2.86)	23.50 (3.68)	23.90 (2.60)	0.430[a]
After	18.00 (1.41) *	19.30 (2.26)	19.90 (1.72)[#]	20.80 (1.75) *,[#]	**< 0.001**[b]
MD (95% CI)	4.50 (3.53, 5.46)	2.70 (1.22, 4.17)	3.60 (1.94, 5.25)	3.10 (1.81, 4.38)	
P value[c]	**< 0.001**	**0.002**	**0.001**	**< 0.001**	
WBC (10^3/μL)					
Before	5.13 (1.96)	5.23 (2.30)	5.28 (14.73)	5.90 (3.78)	0.903[a]
After	6.86 (1.49) *	5.72 (1.69)	6.27 (1.07)[#]	5.75 (1.41) *,[#]	**0.011**[b]
MD (95% CI)	-1.73 (−3.03, −0.42)	-0.49 (- 1.12, 0.14)	-0.99 (−1.41, −0.56)	0.15 (−1.81, 2.11)	
P value[c]	**0. 01**	0.11	**0.01**	0.86	
Lymphocytes (10^3/μL)					
Before	0.97 (0.324)	1.25 (0.36)	1.17 (0.40)	1.42 (0.36)	0.070[a]
After	2.39 (0.74) *	1.85 (0.40)	1.76 (0.34)[#]	1.71 (0.41) *,[#]	**< 0.001**[b]
MD (95% CI)	-1.41 (−1.88, −0.94)	-0.60 (- 0.74, −0.45)	-0.58 (−0.71, −0.45)	-0.29 (−0.41, −0.17)	
P value[c]	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	
Hb (g/dL)					
Before	12.94 (1.97)	12.02 (1.53)	13.34 (1.05)	12.09 (1.57)	0.179[a]
After	12.83 (1.53)	12.08 (1.21)	13.25 (0.88)	12.19 (1.29)	0.747[b]
MD (95% CI)	0.11 (−0.38, 0.60)	-0.06 (−0.39, 0.27)	0.09 (−0.11, 0.29)	-0.10 (−0.46, 0.26)	
P value[c]	0.62	0.69	0.35	0.55	
Platelets (10^3/μL)					
Before	215.50 (62.97)	207.40 (56.04)	182.40 (44.20)	200.40 (52.07)	0.572[a]
After	223.0 (71.92)	217.70 (62.66)	183.60 (42.62)	193.10 (41.46)	0.159
MD (95% CI)	-7.50 (−26.44, 11.44)	-10.30 (- 18.83, −1.76)	-1.20 (−4.79, 2.39)	7.30 (−9.90, 24.50)	
P value[c]	0.394	0.066	0.470	0.362	
LDH (U/L)					
Before	623.70 (147.99)	668.30 (202.24)	615.10 (226.65)	608.00 (99.38)	0.870[a]
After	299.90 (95.75) *	385.00 (107.39)	377.50 (127.53)	485.30 (154.57) *	**0.002**[b]
MD (95% CI)	323.80 (228.43, 419.16)	283.30 (179.72, 386.87)	237.60 (144.51, 330.68)	122.70 (6.18, 239.21)	
P value[c]	**< 0.001**	**< 0.001**	**< 0.001**	**0.041**	
ESR (mm/hr)					
Before	77.70 (18.89)	75.00 (21.82)	56.50 (19.02)	60.60 (14.80)	0.039[a]
After	20.50 (7.61) *	30.50 (10.86)	25.70 (9.39)	22.00 (7.98) *	**0.011**[b]
MD (95% CI)	57.20 (44.55, 69.84)	44.50 (34.89, 54.10)	30.80 (21.43, 40.16)	38.60 (26.44, 50.75)	
P value[c]	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	
hs-CRP (mg/l)					
Before	94.75 (21.43)	82.60 (28.98)	65.00 (29.66)	73.00 (34.04)	0.136[a]
After	36.80 (6.84)	45.10 (18.69)	22.10 (23.86) *	38.90 (23.86) *	**0. 042**[b]
MD (95% CI)	57.95 (46.24, 69.65)	37.50 (22.29, 52.70)	42.90 (17.38, 48.41)	34.10 (11.20, 56.99)	
P value[c]	**< 0.001**	**< 0.001**	**< 0.001**	**0.008**	
IL-1β (pg/ml)					
Before	13.23 (4.49)	17.05 (9.29)	14.47 (6.23)	14.61 (5.94)	0.642[a]
After	9.93 (2.17)	11.92 (5.45)	9.98 (4.02) *	12.58 (5.48) *	**0.001**[b]
MD (95% CI)	4.30 (3.42, 7.17)	5.13 (1.74, 8.51)	4.49 (1.92, 7.05)	2.03 (0.94, 3.11)	
P value[c]	**0.003**	**0.008**	**0.003**	**0.002**	
IL-6 (pg/ml)					
Before	15.85 (11.90–23.25)	13.00 (10.07–45.42)	19.25 (14.80–67.45)	12.95 (9.15–69.72)	0.494[d]
After	8.10 (6.62–11.80)	8.35 (7.72–25.12)	10.58 (8.22–32.80)	11.80 (7.85–45.95)	0. 063[b]
P value[e]	**0.005**	**0.001**	**0.005**	0.131	

Values are expressed as mean (SD). In each row, the mean value with different superscript *and # are significantly differences (P < 0.05) between each group with placebo group in sidak test

[a] One-way analysis of variance (ANOVA).

[b] ANCOVA adjusted for age, sex, and baseline values.

[c] Paired-samples *t*-test.

[d] Non-parametric Kruskal–Wallis test.

[e] Non-parametric Wilcoxon signed-rank test.

terms of age, body mass index (BMI), the severity of the disease, and common symptoms of COVID-19 (Table 1).

At baseline, there were no significant differences among four groups, for the clinical and biochemical parameters. At the end of the study, the O2sat and RR were statistically different between the groups ($p < 0.001$). Compared to the placebo, boron citrate supplementation alone or in combination with OEA led to significant increases in O2sat ($p = 0.001$ and $p = 0.007$, respectively) and RR ($p = 0.003$ and $p = 0.001$, respectively) after adjusting for the potential confounding factors, while OEA supplementation alone had no significant effects on theses parameters. White blood cells (WBCs) and lymphocyte count were significantly different ($p = 0.011$ and $p < 0.001$, respectively) after the intervention comparing the four groups. Significant increases in WBCs and lymphocyte count were observed in the boron citrate and combined groups ($p < 0.001$), compared to the placebo, at the end of the study. No significant within-or between-group differences were observed for platelet count and hemoglobin concentrations. Between-group analyses adjusted for confounding variables showed a significantly lower levels of LDH and ESR in the boron group compared to the placebo, post-intervention. Boron citrate supplementation led to a

Table 3
Common COVID-19 symptoms among patients at the end of the study.

	Boron (n = 10)	OEA (n = 10)	Boron + OEA	Placebo (n = 10)	P value
No fever	10 (100)	9 (90)	9 (90)	9 (90)	0.782
No cough	10 (100)	9 (90)	8 (80)	2 (20)	**< 0.001**
No fatigue	6 (60)	4 (40)	3 (30)	0 (0)	**0.036**
No shortness of breath	10 (100)	8 (80)	9 (90)	4 (40)	**0.041**
No mylagia	7 (70)	8 (80)	8 (80)	4 (40)	**0.341**
No sore throat	10 (100)	10 (100)	10 (100)	9 (90)	0.380

OEA, oleoylethanolamide.
Values are presented as number (%).
P value for chi-square ($\chi 2$) test.

significant decrease in serum LDH ($p = 0.026$) and ESR ($p = 0.014$), compared with other groups. Furthermore, we found that boron citrate in combination with OEA led to a significant reduction in the hs-CRP and IL-1β concentrations ($p = 0.031$ and $p = 0.027$, respectively). Additionally, serum concentration of IL-6 was marginally different between the groups, at end of trial ($P = 0.063$) (Table 2).

At the end of the study, the most common symptoms of COVID-19 including cough ($p < 0.001$), fatigue ($p = 0.036$), shortness of breath ($p = 0.041$), and myalgia ($p = 0.341$) significantly improved in the supplemented groups, compared to the placebo ($p < 0.05$), however, no significant differences were observed among four groups in terms of fever and sore throat (Table 3).

7. Discussion

To the best of our knowledge, this is the first randomized clinical trial investigating the effects of boron citrate plus OEA supplementation in patients with COVID-19. Our results demonstrated that supplementation with boron citrate alone or in combination with OEA significantly improved O2sat and RR. Moreover, significant increases in white blood cell and lymphocyte count were observed in the boron citrate and combined groups, at the end of the study. However, we did not observe significant differences among groups post-intervention, in terms of hemoglobin concentrations and platelet count, in our study. Boron citrate supplementation led to a significant decrease in serum levels of LDH and ESR. Furthermore, boron citrate in combination with OEA resulted in a significant reduction in hs-CRP and IL-1β concentrations. Moreover, serum concentration of IL-6 was marginally different ($P = 0.063$) between the groups, at end of the trial (Table 2). Cough, fatigue, shortness of breath, and myalgia significantly improved in the supplemented groups at the end of the study (Table 3).

It has been demonstrated that most patients infected with COVID-19 exhibit leukocytopenia, lymphopenia, and elevated levels of inflammatory biomarkers in the primary form of the disease. With disease progression, increasing levels of LDH, ESR, creatine kinase, and creatinine may also be observed [36,37]. COVID-19 infection leads to the release of pro-inflammatory cytokines, which are a critical component of the innate immune response and play a major role in clearing viral infections. However, the dysregulated release of pro-inflammatory cytokines causes a cytokine storm, which can lead to cell death as well as tissue damage [38,39]. Boron-containing compounds have antioxidant and anti-inflammatory activity. The importance of dietary boron in improving antioxidant defense mechanisms, thereby ameliorating oxidative stress has been suggested in previous works [40]. Boron effectively ameliorated acrylamide-induced oxidative stress, inflammation, altered biochemical parameters and alleviated tissue damage by preventing the consumption of antioxidant enzymes and inhibiting lipid peroxidation in rats [10]. Recent studies have demonstrated that boron-containing compounds stimulate the release of chemical mediators of inflammation, which are important in host defense against infections. In mice stimulated with lipopolysaccharide (LPS), administration of borax, a salt of BA, induced lymphocyte proliferation and increased the release of pro-inflammatory mediators, cytokines and nitric oxide [19]. The ability of boron to regulate the inflammatory responses has also been reported in human. In the areas of the world where boron intakes were 3–10 mg/day, the estimated incidence of arthritis ranges from 0% to 10%. While, in areas where the boron intakes were 1.0 mg or less/ day, the estimated incidence of arthritis ranges from 20% to 70%, demonstrating the protective role of boron in inflammatory processes [41]. The virus-inhibiting activity of boron-containing compounds has also been reported. In this regard, emerging evidence indicates that boron-based drugs could block severe fever with thrombocytopenia syndrome (SFTSV) infection by affecting virus infectivity, replication, and release [42]. The inhibitory effect of boromycin as an organoboron compound on the replication of HIV has also been reported [26].

It has been well established that OEA exerts anti-inflammatory effects by enhancing PPAR-α expression and by suppressing the expression of pro-inflammatory factors such as cyclooxygenase-2 (COX-2), IL-6, IL-1β, CRP, tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), and toll like receptors-4 (TLR4)-mediated nuclear factor kappa B (NF-κB) signaling cascade [32,35,43,44]. In a recent clinical trial on obese patients, OEA supplementation significantly reduced serum levels of IL-6 and TNF-α [45]. In addition, in obese patients with fatty liver, OEA treatment resulted in a significant decrease in the expression level of inflammatory markers including NF-κB and IL-6 and a significant increase in the expression level of IL-10 as an anti-inflammatory cytokine [32]. OEA also acts as scavenger for ROS and increase anti-oxidative enzymes [46]. OEA is derived from the monounsaturated fatty acid, oleic acid (OA) [47]. It has been demonstrated that angiotensin receptors can be inhibited by unsaturated fatty acids and their metabolites such as OA [35]. In poliovirus-infected HeLa cells, incorporation of OA into membranes resulted in increased membrane fluidity, making these membranes nonfunctional for poliovirus RNA replication, and blocked viral RNA synthesis. Additionally, OA could strongly protect cells from the cytopathogenic effect induced by poliovirus [48]. All the above mentioned points indicate that the beneficial effects of boron citrate and OEA in our patients might be mediated through their anti-inflammatory, antioxidant, and immunomodulatory effects.

There are some limitations in the present clinical trial to be considered in data interpretation such as small sample size and short duration of the study. Therefore, further clinical trials with larger sample sizes and longer duration are warranted. Moreover, different dosages of boron citrate and OEA are recommended to be considered in future studies. Furthermore, a lack of measuring OEA and boron citrate serum levels due to financial restrictions, that was the most appropriate method for measuring the compliance of the study patients, was another limitation of this study. However, this study appears to be the first human trial assessing therapeutic effects of boron citrate and OEA supplementation in patients with COVID-19.

8. Conclusion

In conclusion, the present clinical trial, for the first time, indicated that supplementation with boron citrate alone or in combination with OEA could improve some clinical and biochemical parameters in COVID-19 patients.

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Declaration of Competing Interest

All authors declare that there is no conflict of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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CRediT authorship contribution statement

The authors' responsibilities were as follows: AO, MM, and FN designed research and contributed to the conception of the project, development of overall research plan, and study oversight. HT wrote the original paper; HT, SP, and NF contributed to the statistical analysis; HT and NA contributed to the final revision of the manuscript. HS and BK were involved in the synthesis of supplements. All authors read and approved the final version of the manuscript.

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